Standardisation of Factor VIII Inhibitor Assays

National Institute for Biological Standards and Control, UK.

A Comparison of the Bethesda and New Oxford Methods of Factor VIII Antibody Assay

Austen, Lechner, Rizza and Rhymes. *Thromb Haemost* 1982; 47: 72-75.

Samples: *Plasma from 8 patients with inhibitors*
Participants: *11 Laboratories*
Assays: *Participants carried out assays on each sample in triplicate, by both the Bethesda and the New Oxford methods*

Inter-laboratory Variability
CVs of Mean of 3 Results

Sample		
	52%	85%
	66%	101%
	62%	60%
	52%	128%
	42%	47%
	38%	90%
	-	-
	78%	66%

Intra-laboratory Variability Range of CVs

Method		
	0.0	37.0
	1.0	65.0

UK-NEQAS Study on Factor VIII:C Inhibitor Assay (Survey 126) 2001

F. E. Preston and T.A.L Woods.

Unpublished Data (Personal Communication)

Samples:

(UK centres only) - Plasma from a haemophiliac with inhibitor which also cross-reacted with porcine factor VIII:C.

(international laboratories) - Plasma from a patient with an acquired inhibitor.

Participants: *60 UK Labs; 18 International Labs.*

Assays: *Participants carried single assays on each sample, by the Bethesda Method (1 Lab - New Oxford method)*

Detection Limit of FVIII:C Inhibitor Assays

UK Laboratories

Lowest limit of detection:

B.U.	0	0.05	0.1	0.2	0.25	0.3	0.4	0.5	0.6	1.0
N	4	1	4	5	1	5	10		1	8

International Laboratories

Lowest limit of detection:

B.U.	0	0.01	0.05	0.1	0.2	0.4	0.5	1.0
N	3	1	1	2	1	2		2

Results 1- Sample 01/10 (Human FVIII:C Inhibitor Assay)

	60
	2.18
	1.15
	47.3
	0.6
	8.0

Results 2 - Sample 01/10 (Porcine FVIII:C Inhibitor Assay)

	18
	1.1
	0.74
	60.5
	0.0
	2.7

Results 3 - Sample 01/10A (Human FVIII:C Inhibitor Assay)

	18	60
	1.15	1.40
	0.94	1.61
	68.4	86.0
	0.41	0.4
	4.0	9.0

Acquired Inhibitor

A Controlled Study to Compare Bethesda Factor VIII Inhibitor Assays NIBSC Wet Workshop

T.W. Barrowcliffe, I.R. Peake and A.D. Curtis.

Unpublished Study **1985**

Samples: *Haemophilic plasma samples with and without inhibitors*

Participants: *16 Participants (UK Haemophilia Centres)*

Assays: *Participants carried out replicate assays repeatedly on each sample, in the presence and absence of inhibitor, by the Bethesda method*

Summary of Results of NIBSC Wet Workshop

- CVs for samples without Inhibitor: 15-26%
- CVs for samples with Inhibitor: 53-80%
- CVs for samples with Inhibitor when incubation stage standardised: 33-43%
- CVs for samples with Inhibitor when incubation + FVIII assay stages standardised: 14-20%
- Intra-operator CVs < Inter-operator CVs

NIBSC

International Collaborative Study to Standardise anti-FVIII Inhibitor Assays

S Raut, D Sands, T Barrowcliffe, NIBSC, UK.

S Kitchen, FE Preston Sheffield, UK.

NIBSC

Study Summary

- 2 samples containing human anti-FVIII monoclonals and 1 sample containing rabbit anti-FVIII polyclonal
- 3 samples from haemophiliacs with inhibitor developed secondary to treatment
- Assayed in multi centre study - 15 centres, 17 sets of assay data using local Bethesda methods (75% - Nijmegen modification)
- Variability of inhibitor assays was assessed
- Could a reference material assist in standardisation of this assay?

Inhibitor results – n = 17

	Inhibitor 1	Inhibitor 2	Inhibitor 3
Mean (Bu/ml)	13.4	5.0	13.0
Range (Bu/ml)	4.1 – 22.0	2.1 – 14.3	6.1 – 17.9
CV %	39.5	52.4	33.7

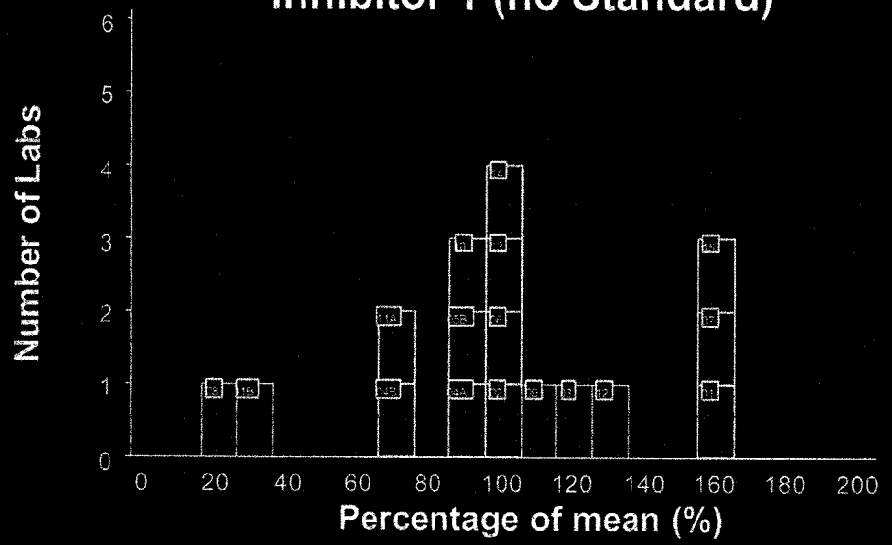
Inhibitor results – n = 17

	99/648 (rPAb)	99/652 (hMAb 1)	99/654 (hMAb 2)
Mean (Bu/ml)	31.5	32.4	35.1
Range (Bu/ml)	23 - 47	19 - 45	20 - 54.8
CV %	26.2	28.9	30.1

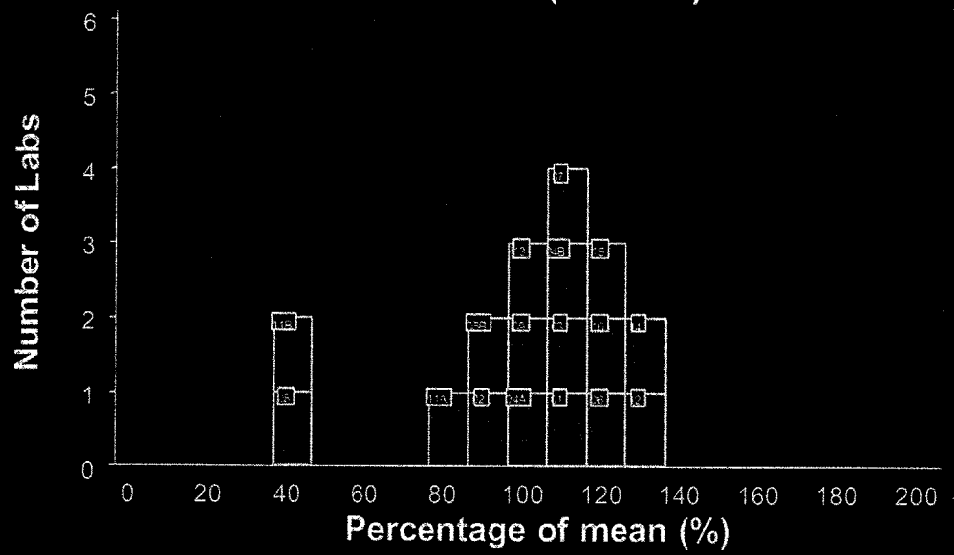
One stage (n= 11) & Chromogenic assay (n = 5)

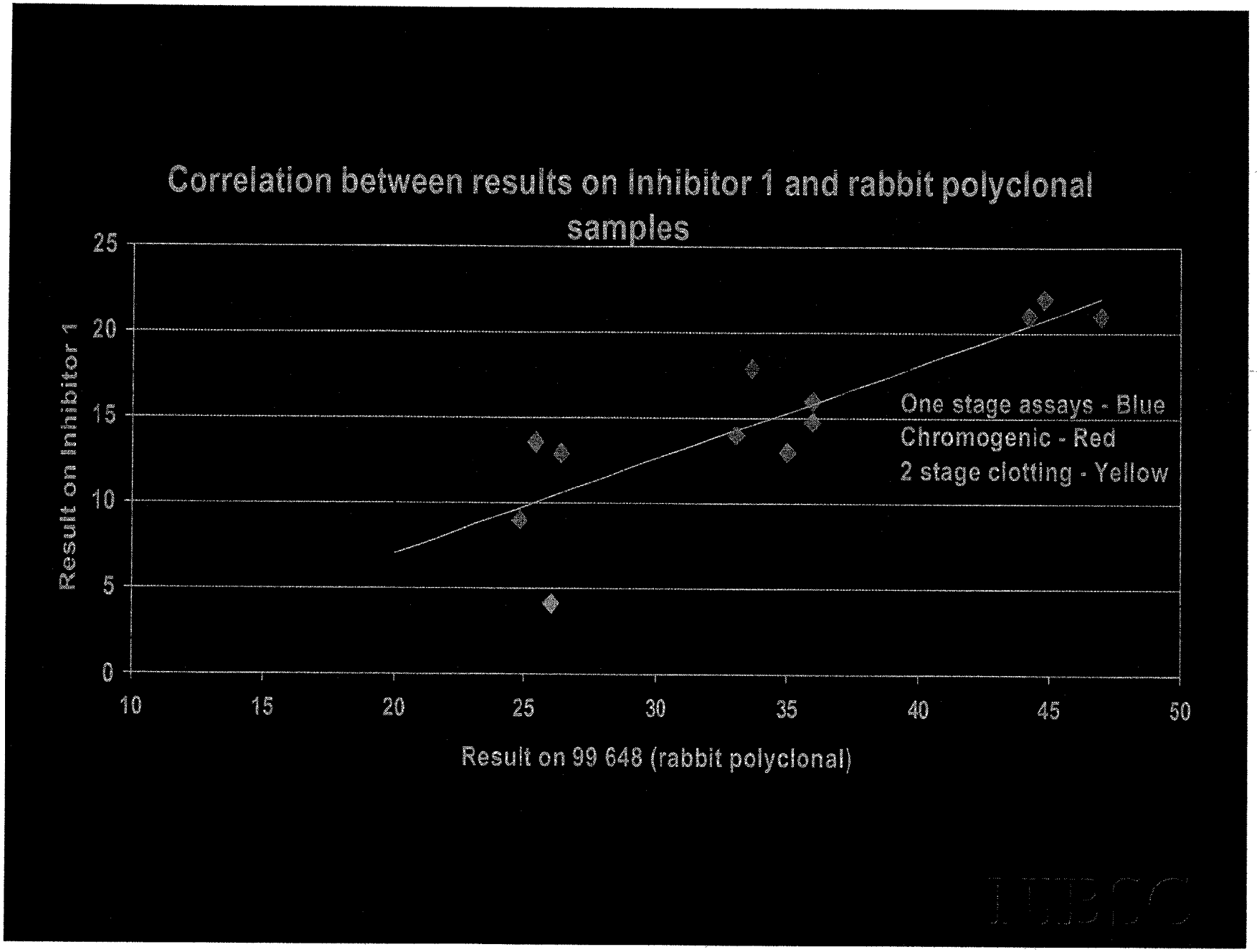
	1	2	3	rPAb	hMAb 1	hMAb 2
One - stage	15.9	5.6	14.8	35.2	32.3	36.0
Chromogenic	9.7	4.4	10.4	24.6	32.7	35.7
Difference		27%			1%	1%

Inhibitor 1 (no Standard)



Inhibitor 1 vs rPAb (99/648) as Standard




Correlation between results on Inhibitor 1 and rabbit polyclonal samples
Result on inhibitor 1
25
20
15
10
5
0
10
15
20
25
30
35
40
45
50
Result on 99 648 (rabbit polyclonal)
One stage assays - Blue
Chromogenic - Red
2 stage clotting - Yellow

Relationship between results on the patient samples and results on the antibody preparations

Correlation coefficient for patient samples against:

- MAb 1 (99/652) – 0.08, 0.21, 0.15 ns
- MAb 2 (99/654) – 0.57 ($p<0.01$), 0.27 and 0.39
- PAb (99/648) – 0.86, 0.60, 0.59 (all $p< 0.01$)

Antibody preparations as reference/standard - effect on CVs

	39.5 %	26.7	47.2	37.3
	52.4 %	33.9	47	42.1
	33.7 %	26.5	38.6	26.1

Conclusions

- High inter- lab variability in inhibitor assays (much greater than assays of FVIII:C)
- Some improvement in CVs between centres using a candidate standard as reference, particularly for the rabbit polyclonal
- Large scale production and fill of reference material(s) with full multi assay, multicentre study?
- If so what levels of Bethesda would be most useful?

Possible Advantages of a Reference Preparation

- To reduce inter-lab CVs.
- To have a useful QC material for labs in clinical studies.
- As a common sample in evaluation of new methodologies.

Feedback

- **Proposal:**

  Seek out feedback from participants (questionnaire from NIBSC ?) before embarking on a larger study.

Points to Consider (Overall) 1

- **High variability**
  - directly due to presence of inhibitor
- **A need for an Inhibitor Standard**
  - Intra-Lab CVs < Inter-Lab CVs
  - Better CVs with Standard

Points to Consider (Overall) 2

- Chromogenic CVs < One-stage CVs (a matrix dilution effect?) May need to modify assessment of residual FVIII:C activity:
  - (a) High dilution
  - (b) Reduced or standardised incubation time

- Time for different FVIII:C assays to complete vary (will affect time for inhibitor to neutralise FVIII)

Points to Consider (Overall) 3

- **Standardise the assays:**

  - Ab dilutions (critical)

  - FVIII deficient plasma
  - Reagents (phospholipid, activator)
  - Order of addition of reagents mixture (assay design)
  - Activation time

Acknowledgements

- Dr Jorgen Ingerslev, Aarhus, Denmark - preparation of rabbit polyclonal antibody to human FVIII
- Dr Marc Jacquemin, Leuven, Belgium - preparation of the monclonals from immortalised Haem A patients B lymphocytes.

Participants

- Dr Armelle, France
- Dr S Raut/ Dr Barrowcliffe NIBSC, UK
- Prof Budde, Germany
- Dr Byrne, USA
- Ms Duncan/Dr Lloyd, Australia
- Prof Hillarp,Sweden
- Prof Ingerslev, Denmark
- Dr Kitchen/Prof Preston, UK
- Dr Ruth Laub, Belgium
- Dr Kotitschke, Germany
- Prof Oswaldson/Ms Frank Sweden
- Ms Riddell/Prof Lee, UK
- Dr Rosen, Sweden
- Dr Rothschild, France
- Dr Sahud, USA
- Dr Van Mourik, Netherlands
- Dr Verbruggen, Netherlands
- Prof Yoshioka, Japan